STRATEGIC DIAGNOSTICS INC.

PENTA EnSys® SOIL TEST SYSTEM

RAPID IMMUNOASSAY SCREEN

User's Guide
Multiple Level Test

This method correctly identifies 95% of samples containing 0.5 ppm pentachlorophenol (PCP). A sample that develops less color than the standard is interpreted as positive. It contains PCP. A sample that develops more color than the standard is interpreted as negative. It contains less than 0.5 ppm PCP.

IMPORTANT NOTICE

This test system should be used only under the supervision of a technically qualified individual who is capable of understanding any potential health and environmental risks of this product as identified in the product literature. The components must only be used for the analysis of soil samples for the presence of pentachlorophenol. After use, the kits must be disposed of in accordance with applicable federal and local regulations.

PENTA RISC SOIL TEST TROUBLESHOOTER GUIDE

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

Wash Step - Lack of vigorous washing may result in false positives or negatives depending on whether the wash error was committed on standard or sample tubes.
Solution make sure that the operator washes four times vigorously.

Pipet Calibration - An out-of-calibration pipet may result in false positives or negatives depending on whether the amount is greater or less than the specified transfer volume.
Solution Check the calibration at least daily and after any extreme mechanical shock (such as dropping). An indication that the pipet is out of calibration is if the gold barrel is loose and will turn. (When set on 30µl there should be about 1/4 of an inch between the white plunger and the end of the clear pipet tip.)

Air bubbles in the pipet - The presence of air bubbles in the pipet tip when transferring extracts may result in false positives or negatives depending on whether the error was committed on standard or sample tubes.
Solution Quickly examine the pipet tip each time an aliquot is withdrawn and go back to the source and take another aliquot to displace the air bubble if necessary.

Mixing - Lack of thorough mixing, when instructed, can cause inconsistent results.
Solution Observe the mixing times in the instructions and mix with sufficient force to ensure homogeneity.

Timing - It is important to follow the timing steps in the instructions carefully. The incubation step in the antibody tubes can vary a bit without harm to the test. The color development step timing is critical and should be no less than 2 minutes and no greater than 3 minutes.

Wiping the Tubes - Wiping of the tubes should be done before they are read in the spectrophotometer because smudges and fingerprints on the tubes can give potentially false negative readings.

Mixing Lot #'s - Never mix lots! Each kit's components are matched together for optimal performance and may give inaccurate results with the components from other kits. Also, the user must NEVER mix components from different types of kits (ex: Petro kit buffer can't be used with a PCP kit.)

Storage and Operating Temperatures. - Temperature requirements are very important and should be strictly adhered to. This test kit should be stored at less than 80°F/27°C, and operated between 55°F/13°C and 90°F/32°C.

Shelf Life - Each kit label contains the kit expiration date. To achieve accurate results, kits must be used prior to expiration.

READ TO AVOID COSTLY MISTAKES

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

SAMPLE DILUTION PROGRAM

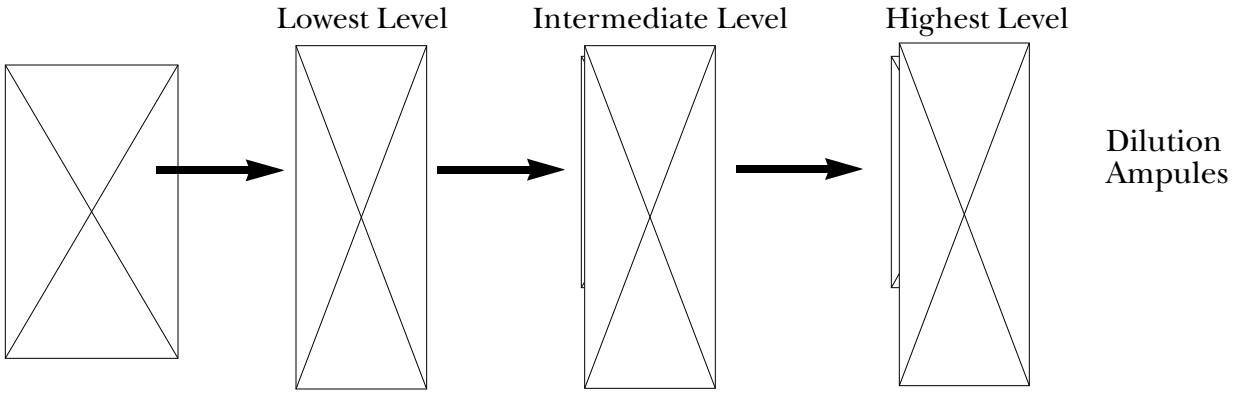
1. The sample dilution procedure on page 6 of the instructions is for 0.5, 5, and 50 ppm detection levels. The following diagram represents the sample dilution procedure for all **other detection levels.**

2. EVERY DILUTION AMPULE PROVIDED MUST BE USED!

If there are any questions concerning the dilution procedure please call Technical Services before running the samples to help avoid costly mistakes.

1-800-242-7472 or 919-941-5509.

EXAMPLE:



Note: Always transfer filtered sample to the dilution labeled with the lowest ppm level and then transfer from it to the next higher level dilution.

WORKSTATION SET-UP

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

WORKSTATION SET-UP FOR 1 SAMPLE AT 3 LEVELS

- 2 Mechanical pipet tips
- Stop solution
- 2 PENTA standard Tubes
- 5 Conjugate tubes
- Substrate A
- Filtration barrel & plunger
- 3 blue buffer tubes
- 5 antibody coated tubes
- Substrate B
- Bulb pipet
- 0.5, 5 and 50 ppm dilution ampules
- Eppendorf Tips

READ BEFORE PROCEEDING

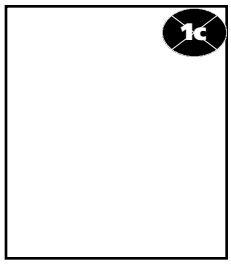
- Follow diagram above to setup workstation.
- Items that you will need that are not provided in the test kit include:
  a permanent marking pen, laboratory tissue (or paper towels), a liquid waste container, and disposable gloves.
- This User's Guide was written for analyzing soil samples for PCP at 0.5, 5 and 50 ppm.
- Label all Eppendorf tips. Tips can be reused for future analyses. Label the first 5mL tip "A", the second tip "B" & the third tip "Stop".

PHASE ONE

EXTRACTION & PREPARATION OF THE SAMPLE

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

WEIGH SAMPLE

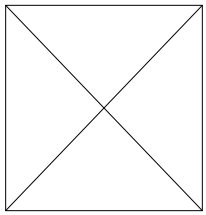


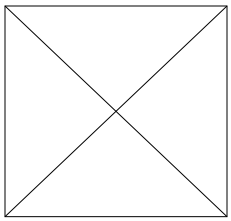
1a Place unused weigh boat on pan balance.

1b Press ON/MEMORY button on pan balance. Balance will beep and display 0.0.

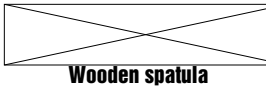
1c Weigh out 10 +/- 0.1 grams of soil.

1d If balance turns off prior to completing weighing, use empty weigh boat to retare, then continue.

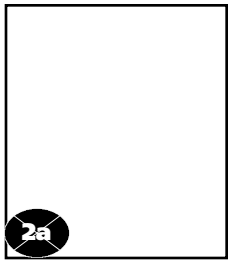

Methanol Extraction Jar


Weigh Boat


Pan balance


Wooden spatula

EXTRACT PCP

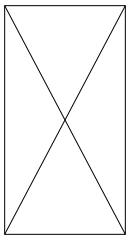


2a Using wooden spatula, transfer 10 grams of soil from weigh boat into extraction jar containing Methanol.

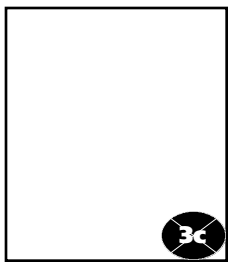
2b Recap extraction jar tightly and shake **vigorously** for one minute.

2c Allow to settle for one minute.

Repeat steps **1a - 2c** for each sample to be tested.


Sample extraction jar

FILTER SAMPLE

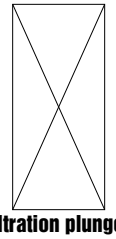


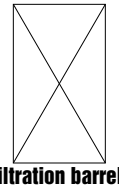
3a Disassemble filtration plunger from filtration barrel.

3b Insert bulb pipet into top (liquid) layer in extraction jar and draw up sample. Transfer **at least** ½ bulb capacity into filtration barrel. **Do not use more than one full bulb.**

3c Press plunger firmly into barrel until adequate filtered sample is available (place on table and press if necessary).

Repeat steps **3a - 3c** for each sample to be tested.


Filtration plunger


Filtration barrel

Bulb pipet

PHASE TWO

SAMPLE AND STANDARD PREPARATION

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

READ BEFORE PROCEEDING

- "Shake tubes" means to thoroughly mix the contents with special care not to spill or splash.

DILUTE AND BUFFER SAMPLE FOR 0.5, 5 & 50 PPM DETECTION LEVELS

See page 3 for other detection levels

4a Flick or tap to get buffer into the bottom of the ampule. Open dilution ampules.

4b Uncap enough conjugate and buffer tubes for **Samples** and **Standards**.

4c Empty two Penta standard tubes into two conjugate tubes.

4d Empty a blue buffer tube into each remaining conjugate tube for samples.

4e Assemble tip onto mechanical pipet.

4f Withdraw 100 µL of sample from filter unit using mechanical pipet and dispense below the liquid level in **0.5 ppm** dilution ampule. Shake for 5 seconds. Wipe mechanical pipet tip.

4g Withdraw 100 µL of diluted sample from **0.5 ppm** dilution ampule and dispense below the liquid level in the **5 ppm** dilution ampule. Shake 5 seconds. Wipe mechanical pipet tip.

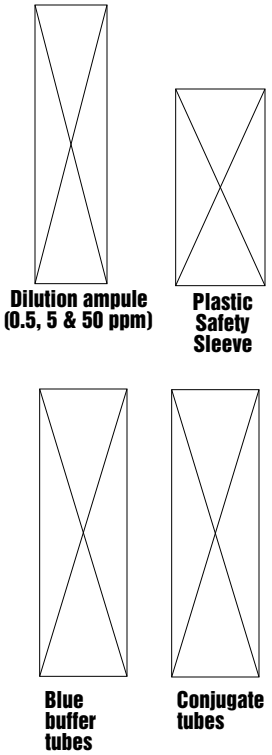
4h Withdraw 100 µL of diluted sample from **5 ppm** dilution ampule and dispense below the liquid level in **50 ppm** dilution ampule. Shake for 5 seconds. Wipe mechanical pipet tip.

4i Withdraw 100 µL of diluted sample from **50 ppm** dilution ampule and dispense below the liquid level in **50 ppm** conjugate tube. Repeat with 5 and .5 ppm test levels.

4j Discard mechanical pipet tip.

Repeat steps **4e - 4i** for each sample to be tested.

4k Mix all conjugate tubes for 5 sec.

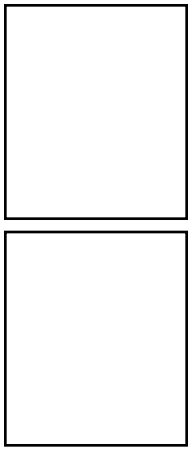


PHASE THREE

THE IMMUNOASSAY

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

TRANSFER FROM CONJUGATE TUBE TO ANTIBODY COATED TUBE



5a. Label the antibody coated tubes with sample identification and test level.

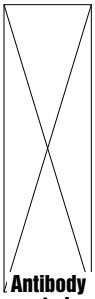
5b. Set timer for 10 minutes.

5c. Working left to right in the workstation:

1. Fit all antibody coated tubes firmly on top of all corresponding conjugate tubes.

2. Start timer and immediately invert **all** connected tube pairs so that the liquid is poured into the antibody coated tubes. Return the tube pairs to the appropriate workstation row making sure the (larger) antibody coated tube is on the bottom.

5d. Disconnect and discard the smaller glass conjugate tubes. [It is not important to worry about drops of liquid adhering to lips of tubes].

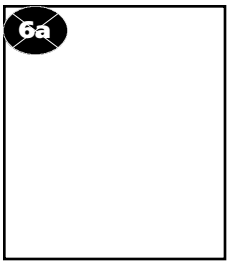


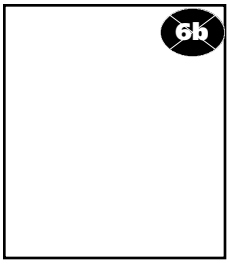
Antibody coated tubes (contained in resealable "zip-seal" aluminized pouch)

WASH PROCEDURE

- Washing must be done vigorously and with force.
- Place nozzle just above antibody coated tube, squeeze bottle to fill each tube with a vigorous stream and empty into liquid waste container.
- The wash solution is a harmless, dilute solution of detergent. Do not hesitate to wash vigorously even if the solution contacts gloved hands.

WASHING

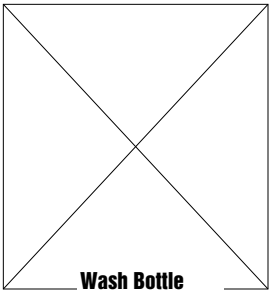




6a. After the 10 minute incubation period, empty antibody coated tubes into liquid waste container.

6b. Wash antibody coated tubes with wash solution by vigorously filling and emptying a total of 4 times.

6c. Tap antibody coated tubes upside down on paper towels to remove excess liquid. Residual foam in the tubes will not interfere with test results.



Wash Bottle

PHASE THREE

THE IMMUNOASSAY

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

COLOR DEVELOPMENT

7a Set the Eppendorf Repeater on 2, assemble the "A" tip and fill with Substrate A (TMB, yellow label).

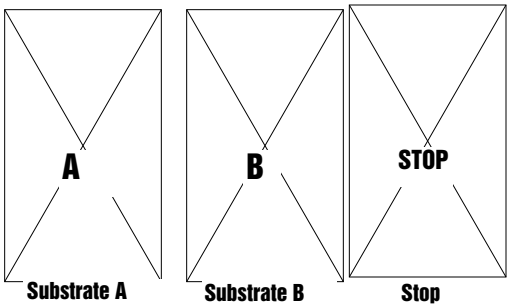
7b Dispense once (200μl) into each antibody coated tube.

7c Set timer for exactly 2 1/2 minutes

7d Assemble "B" tip, fill with Substrate B, (H_20_2 , green label) start timer, and dispense once (200μl) into each antibody coated tube.

7e Shake all tubes for 5 seconds. Solution will turn blue in some or all antibody coated tubes.

7f Assemble "Stop" tip, fill with Stop Solution (red label), and stop reaction at end of 2 1/2 minutes by dispensing once (200μl) into each antibody coated tube.

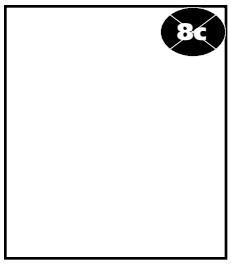


PHASE FOUR

INTERPRETATION

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

SELECT CONSERVATIVE STANDARD



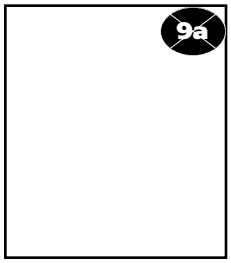
8a Wipe outside of all antibody coated tubes.

8b Place both **Standard** tubes in photometer.

8c Switch tubes until the photometer reading is negative or zero. Record reading. If reading is greater than - 0.3 in magnitude, results are outside of QC limits. Retest the sample(s).

8d Remove and discard tube in right well. The tube in the left well is the conservative standard.

INTERPRET RESULTS



9a Place **0.5 ppm** sample tubes in right well of photometer and record reading.

If photometer reading is **negative** or zero, PCP is present.

If photometer reading is **positive**, concentration of PCP is less than **0.5 ppm**.

9b Place **5 ppm** tube in right well of photometer and record reading shown on display.

If photometer reading is **negative** or zero, PCP is present.

If photometer reading is **positive**, concentration of PCP is less than **5 ppm**.

9c Same as above for 50ppm.

QUALITY CONTROL

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

How It Works

Standards, Samples, and color-change reagents are added to test tubes coated with a chemical specific to pentachlorophenol. The concentration of pentachlorophenol in an unknown **Sample** is determined by comparing its color intensity with that of a **Standard**.

Note: Pentachlorophenol concentration is inversely proportional to color intensity; the lighter the color development of the sample, the higher the concentration of pentachlorophenol.

Quality Control

Standard precautions for maintaining quality control:

- Do not use reagents or test tubes from one Test System with reagents or test tubes from another Test System.
- Do not use the Test System after its expiration date.
- Each analysis must include 2 **Standards**, with no more than a total of 12 antibody coated tubes.
- Do not exceed incubation periods prescribed by the specific steps.
- Results may not be valid if photometer reading for **Standards** exceeds 0.3 in magnitude.

Storage and Handling Precautions

- Wear protective gloves and eyewear.
- Store kit at room temperature and out of direct sunlight (less than 80°F).
- Keep aluminized pouch (containing unused antibody coated tubes) sealed when not in use.
- If liquid from the extraction jar, or PCP Standard comes into contact with eyes, wash thoroughly with cold water and seek immediate medical attention.
- Operate test at temperatures greater than 13˚ C/55˚ F and less than 32˚ C/90˚ F.
- After use, dispose of kit components in accordance with applicable federal and local regulations.

System Description

Each Penta RIS^{C}® Soil Test System contains enough material to perform twelve complete tests, at two different test levels.

The Penta RIS^{C}® Soil Test is divided into four phases. The instructions and notes should be reviewed before proceeding with each phase.

Hotline Assistance

If you need assistance or are missing necessary Test System materials, call toll free: 1-800-242-RISC (7472).

Validation and Warranty Information

Product claims are based on validation studies carried out under controlled conditions. Data has been collected in accordance with valid statistical methods and the product has undergone quality control tests of each manufactured lot.

Pentachlorophenol -free soil and soil containing 0.5 ppm of pentachlorophenol were tested with the EnSys Penta RIS^{C}® analytical method. The method correctly identified 95% of these samples.

The company does not guarantee that the results with the Penta RIS^{C}® Soil Test System will always agree with instrument-based analytical laboratory methods. All analytical methods, both field and laboratory, need to be subject to the appropriate quality control procedures.

EnSys, Inc. warrants that this product conforms to the descriptions contained herein. No other warranties, whether expressed or implied, including warranties of merchantability and of fitness for a particular purpose shall apply to this product.

EnSys, Inc. neither assumes nor authorizes any representative or other person to assume for it any obligation or liability other than such as is expressly set forth herein.

Under no circumstances shall EnSys, Inc. be liable for incidental or consequential damages resulting from the use or handling of this product.

REPEATER PIPET & MECHANICAL PIPET

READ ALL INSTRUCTIONS BEFORE PROCEEDING WITH THE TEST

HOW TO OPERATE THE REPEATER PIPET

To Set Or Adjust Volume

To determine the pipetting volume, the dial setting (1-5) is multiplied by the minimum pipetting volume of the tip.

To Assemble Pipet Tip

Slide filling lever down until it stops. Then raise the locking clamp and insert the tip until it clicks into position. Be sure the tip plunger is fully inserted into the barrel before lowering the locking clamp to affix the tip in place.

To Fill Tip

With tip mounted in position on pipet, immerse end of tip into solution. Slide filling lever upward slowly.

To Dispense Sample

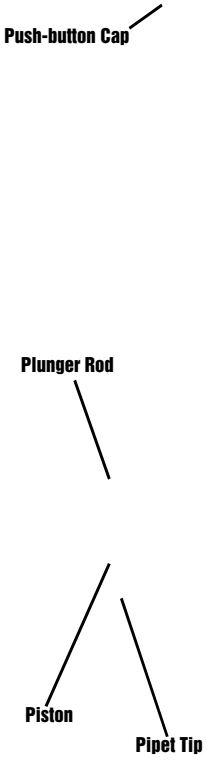
Check the volume selection dial to ensure pipetting volume. Place tip inside test tube so that tip touches the inner wall of tube. Completely depress the pipetting lever.

To Eject Tip

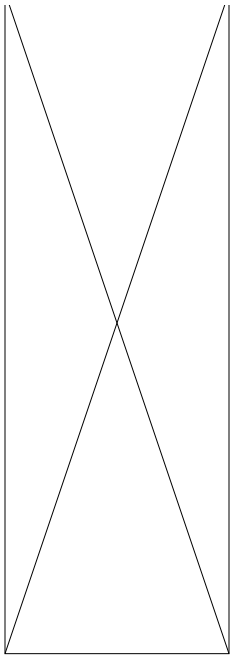
Empty tip of any remaining solution into appropriate container. Raise locking clamp upward, and remove the tip.

For additional information regarding operation and use of repeater, please refer to your Repeater pipet manual.

Mechanical Pipet



Repeater Pipet



HOW TO OPERATE THE MECHANICAL PIPET

To Set Or Adjust Volume

Remove push-button cap and use it to loosen volume lock screw. Turn lower part of push-button to adjust volume up or down. Meter should read "100". Tighten volume lock screw and replace push-button cap.

To Assemble Pipet Tip

Slide larger mounting end of pipet tip onto end of pipet. Holding tip in place, press push-button until plunger rod enters pipet tip. **Ensure no gap exists between piston and plunger rod**.

To Withdraw Sample

With tip mounted in position on pipet, press push-button to first stop and hold it.

Place tip at bottom of liquid sample and slowly release push-button to withdraw measured sample. Ensure that no bubbles exist in liquid portion of sample. If bubbles exist, dispense sample and re-withdraw sample.

To Dispense Sample

Place tip into dispensing vessel (immersing end of the tip if vessel contains liquid) and slowly press push-button to first stop. (Do not push to second stop or tip will eject).

Remove tip from vessel and release push-button.

To Eject Tip

Press push-button to second stop. Tip is ejected.

For additional information regarding operation and use of pipet, please refer to your pipet manual.

ON-SITE QUALITY CONTROL/QUALITY ASSURANCE RECOMMENDATIONS
EnSys RIS^{C}® TEST SYSTEM

Please read the following before proceeding with field testing.

SAMPLING

The result of your screening test is only as valid as the sample that was analyzed. Samples should be homogenized thoroughly to ensure that the 10 grams you remove for field testing is representative of the sample as a whole. All other applicable sample handling procedures should be followed as well.

PRIOR TO TESTING SAMPLES

Carefully follow the instructions in the User's Guide included with every test kit. This is the key element in obtaining accurate results. In addition, store your unused test kits at room temperature and do not use them past their expiration date (see label on each test kit).

INTERNAL TEST QC

Two standards are analyzed with each sample to provide internal test system quality control. With both standards inserted in the photometer, a valid test is indicated when the magnitude of the displayed number (irrespective of the sign, + or -) is less than the value given in the User's Guide. Test runs resulting in a greater number should be repeated to ensure valid conclusions.

QA/QC

The validity of field test results can be substantially enhanced by employing a modest, but effective QA/QC plan. EnSys recommends that you structure your QA/QC plan with the elements detailed below. These have been developed based on the data quality principles established by the U.S. Environmental Protection Agency.

A. **Sample Documentation**
1. Location, depth
2. Time and date of collection and field analysis

B. **Field analysis documentation** - provide raw data, calibration, any calculations, and final results of field analysis for all samples screened (including QC samples)

C. **Method calibration** - this is an integral part of SDI RIS^{C}® immunoassay tests; a duplicate calibration is performed for each set of samples tested (see the instructions in the User's Guide)

D. **Method blank** - field analyze the contents of an unused extraction jar

E. **Site-specific matrix background field analysis** - collect and field analyze uncontaminated sample from site matrix to document matrix effect

F. **Duplicate sample field analysis** - field analyze duplicate sample to document method repeatability; at least one of every 20 samples should be analyzed in duplicate

G. **Confirmation of field analysis** - provide confirmation of the quantitation of the analyte via an EPA-approved method different from the field method on at least 10% of the samples; choose at least two representative samples testing above the action level; provide chain of custody and documentation such as gas chromatograms, mass spectra, etc.

H. **Performance evaluation sample field analysis (optional, but strongly recommended)** - field analyze performance evaluation sample daily to document method/operator performance

I. **Matrix spike field analysis (optional)** - field analyze matrix spike to document matrix effect on analyte measurement

FURTHER QUESTIONS?

EnSys technical support personnel are always prepared to discuss your quality needs to help you meet your data quality objectives.

Data for PENTA RISC® Soil Test

Operator: ---------- Date: ---------- Location: ----------

Sample ID	ΔOD	OD sample	Interpretation	OD sample	Interpretation	OD sample	Interpretation	Comments
	Standards	_____ ppm		_____ ppm		_____ ppm		